CYTOTAXONOMIC STUDIES IN THE GENUS *URGINEA* STEIN IN WEST AFRICA. II. KARYOTYPE EVOLUTION IN *URGINEA ALTISSIMA* (L.) BAKER[1]

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ABSTRACT

Mitotic and meiotic studies were carried out on *Urginea altissima* with $2n = 22$. At pachynema-diakinesis pollen mother cells had 9 (1.61%), 10 (93.9%), or 11 (5.0%) chromosome bodies. Pollen viability was 93.56%. Statistical analysis showed a strong correlation between the proportion of PMCs with (a) 9 bodies and those with anaphase bridges; (b) 10 bodies and those with normal anaphase movements, and pollen viability; and (c) 11 bodies and those with excluded chromosomes. The eleventh homologue was associated with the second largest homologue in the PMCs with 10 bodies. This association is specific and ensures the successful transmission of the eleventh homologue to the spores. Failure of these two homologues to associate always results in the breakdown of normal meiotic behavior. In mitosis, the eleventh pair behaved normally. Hence the correct diploid number is 20 + 2 homologous fragments.

Urginea Stein is a genus of bulbous geophytes in the Liliaceae. It is represented by the basic chromosome numbers of 5 and 7 (Darlington & Wylie, 1955; Jones & Smith, 1967). The known tropical African members have a somatic complement of 14 (a species endemic to Madagascar), 20 or 20 + 0–6 B chromosomes (Jones & Smith, 1967), or 22 (Oyewole, 1975b). The two West African species with a somatic chromosome number of 22, viz., *U. altissima* (L.) Baker sensu stricto and *U. gigantea* (Jacq.), represent a departure from the established basic chromosome numbers. They therefore offer a novel opportunity for investigating and understanding the evolution of genetic systems in the genus, possibly opening up avenues for understanding the mode of speciation in a family known to contain groups of morphologically similar and closely related taxa. Hence the behavior of the chromosomes of *U. altissima* sensu stricto has been investigated in this work.

MATERIALS AND METHODS

Sample collections from natural populations of *U. altissima* sensu stricto were cultivated in experimental gardens at Ibadan and later at Ilorin. These were investigated cytologically, using root tip squashes for mitosis and flower bud (anther) squashes for meiosis, both pretreated for one hour in sat. aq. solution of p-dichlorobenzene. Twenty-five plant stands from various population locations in western Nigeria were investigated. Conventional methods (Darlington & La Cour, 1942; Marenah & Holden, 1967) of squash preparation were employed. Two percent acetic orcein was used. Viability of pollen from undehisced anthers extracted from open flowers was estimated by observing the ability of grains to stain in 1% acetocarmine within two to three minutes.

RESULTS

All 22 chromosomes of the somatic complement behave normally at mitosis (Fig. 1). None shows differential staining except at the centromeric points. They all move normally at anaphase; the number and form, from one cell generation to another (and indeed in different tissues), remain consistent (Oyewole, 1975b).

The pairing behavior at meiosis, as well as pollen viability estimates (Table 1), shows that the taxon has a stable chromosomal system, with a high average pollen viability of 93.56%. However, meiotic formations of 9, 10 or 11 chro-

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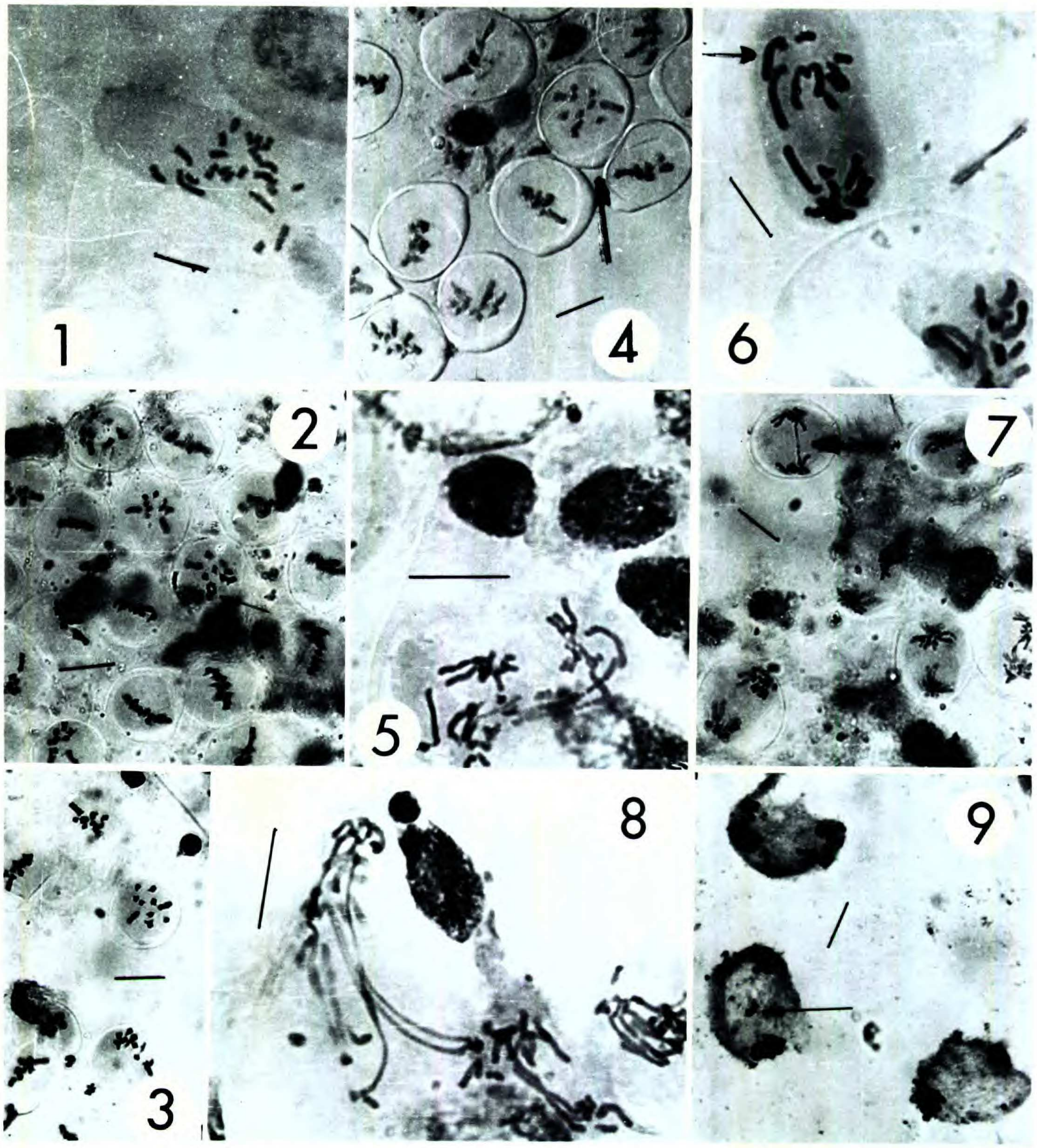

FIGURES 1–9.—1. Somatic complement of 22 chromosomes.—2. PMCs at Metaphase I–Anaphase I, one of which has nine chromosome bodies. The hexavalent is arrowed.—3. PMCs at Metaphase I showing 10 bodies.—4. PMCs at Metaphase I. Arrow indicates the PMC showing 11 bivalents.—5. A PMC with Anaphase I bridge persisting into MII.—6. A PMC at Anaphase I with normal movement. There are 10 chromosomes per group. Second large chromosome, arrowed in one group, shows a conspicuous second arm (cf. Fig. 1).—7. PMC (arrowed) at AI with a chromosome bridge (dicentric).—8. Persistent AI and AII bridges.—9. Telophase I PMCs. One has excluded chromosomes (arrowed).—Each bar (——) represents 10 μm.

mosome bodies per pollen mother cell (PMC) during the first prophase to metaphase were observed (Figs. 2–4). The nine bodies consist of one hexavalent and eight bivalents; the 10 bodies consist of nine bivalents and one quadrivalent; the 11 bodies are all bivalents. These formations occur at frequencies of 1.61%, 93.39%, and 5.0%, respectively. Figures 5–7 demonstrate first anaphases: anaphase bridges (Fig. 5), normal movement (Fig. 6), and lagging and an excluded pair of chromosomes (Fig. 7). These occur at frequencies of 1.23%, 93.51%, and 5.44% respec-

TABLE 1. Meiotic behavior of *U. altissima* Baker sensu stricto.

	Total PMCs				Examined at									
					AI–TI				AII–TII			Pollen Viability		
	Diplonema–MI													
	Chromosome Bodies Formed													
	9	10	11	Total PMCs	Normal Anaphase Movement	Anaphase Bridge Formation	Telophase with Excluded Chromosomes	Total PMCs	Normal Anaphase Movement	Anaphase Bridge & Excluded Chromosomes	Total PMCs	Normal Pollen	Aborted Pollen	Total Pollen Examined
Number	10	579	31	620	533	7	31	570	309	21	330	4,678	322	5,000
%	1.61	93.39	5.0	100	93.51	1.23	5.44	100	93.64	6.36	100	93.56	6.44	100

PMCs = pollen mother cells; MI = metaphase I; AI = Anaphase I; TI = Telophase I; AII = Anaphase II; TII = Telophase II.

TABLE 2. Results of χ^2 to test the null hypothesis that:

(1) % pollen mother cells (PMCs) showing 10 chromosome bodies, normal anaphase movements, and normal pollen are equal (ratio 1 : 1 : 1 : 1);

(2) % PMCs with 9 chromosome bodies and % PMCs with Anaphase I bridge formation are equal (ratio 1 : 1);

(3) % PMCs with 11 chromosome bodies and % PMCs with Telophase I excluded chromosomes are equal (ratio 1 : 1); and

(4) Total % PMCs with 9 and 11 chromosome bodies, % PMCs with AI bridge formation and TI excluded chromosomes, % PMCs with AII bridge formation and TII excluded chromosomes, and % aborted pollen grains are equal.

	χ^2 Value	Probability Value
(1)	0.000605	$P > 99\%$
(2)	0.0508	$90\% > P > 80\%$
(3)	0.01854	$90\% > P > 80\%$
(4)	0.0096	$98\% > P > 95\%$

Level of significance is set at 0.05. The null hypothesis is proved in each case.

tively. Figures 8 and 9 show Anaphase II (AII) diagonal bridge and Telophase II (TII) excluded chromosomes, which also occur at a total frequency of 6.36% while normal AII movement occurs at a frequency of 93.64%. Statistical analyses of these data (Table 2) show that pollen viability percentage and the frequencies of AI and AII normal movements and the formation of 10 chromosome bodies per PMC during the first prophase to metaphase (93.56%, 93.64%, 93.51%, and 93.39%, respectively) are not significantly different; that the frequency of formation of nine bodies per PMC is not significantly different from the frequency of occurrence of laggards and excluded chromosomes at TI; and that the total frequencies of formation of nine and 11 bodies, AI bridge formation and TI chromosome exclusion, AII diagonal bridge formation and TII chromosome exclusion, and percentage pollen abortion are all not significantly different.

In the formation of nine bodies, the hexavalent is an association involving three small pairs including the eleventh homologue, whereas the quadrivalent in the formation of 10 bodies is an association of the eleventh homologue with the second largest pair (cf. chromosome bodies in Figs. 2–4). When this association occurs, the first

and second large pairs become about equal in length at MI (see Fig. 4; see also mitotic chromosome lengths of the two large and eleventh homologues, Oyewole, 1975b). This means that when the eleventh homologue forms a bivalent of its own (in 11 separate bivalents), or associates with any homologues other than the second largest, meiotic behavior becomes erratic and meiotic products are inviable.

Discussion

Some representatives of *Urginea* in other areas have been shown to have B chromosomes (De Wet, 1957; Jones & Smith, 1967), but accessory chromosomes have not been reported in any of the west tropical African materials. Where they have been reported, these accessory chromosomes are known to be different from the autosomal members of their complement. They are heterochromatic, their numbers vary within populations, their transmission at mitosis is not regular, and at meiosis there has not been any mechanism of transmission comparable to that shown in *U. altissima,* where association of the extra pair with a specific pair of autosomes is the mechanism for successful transmission of the extra chromosomes into the spores and thereby into the next generation of the plant. Hence the extra pair of chromosomes in *U. altissima* cannot be seen as essentially inert as in the cases of the B chromosomes reported in other species.

The formation of a hexavalent involving the extra pair and two other pairs may indicate some genetic affinities between the three pairs involved (Battaglia, 1964), or show the probable origin of the extra pair (Wedberg et al., 1968). However, the formation of an association between the extra pair and a specific pair, viz., the second large pair, with a much higher frequency than in the hexavalent formation, and leading to successful meiotic behavior, may identify the true origin of the extra pair. This extra pair cannot be regarded as accessory because it is indistinguishable from the other pairs in mitotic behavior and structure (cf. chromosomes in *Clarkia unguiculata,* and *C. williamsonii* in Mooring, 1960, and Wedberg et al., 1968, respectively). The extra pair behaves normally at mitosis as in the large B chromosomes of *Brachychome lineariloba* (Carter & Smith-White, 1972), but its successful transmission from one generation of the plant to another depends upon a meiotic mechanism of its association with a specific pair of autosomes in the complement. Hence, it differs from the B chromosomes of *B. lineariloba.*

The origin of the extra pair is undoubtedly betrayed in its association with the second large pair—only such association leads to viable spore formation. The mode of origin of the extra pair is probably by fragmentation at a heterochromatic region along the second (short) arm of the parent (second large) autosomal pair. This is why the point of breakage on the otherwise acentric fragment is able to exercise secondarily a spindle-fiber organizing function at mitosis for effective polar movement—and, hence, normal autosomal behavior—but which is incidentally too weak to effectively organize the movement of the fragments at meiosis whenever they form a bivalent of their own. They then remain excluded on the equatorial plane after polar movement of the other chromosomes.

Again, in the formation of 11 normal bivalents, incomplete pairing or total asynapsis in the extra pair, which would lead to early repulsion of its members prior to anaphase movement, was not observed at all. That is, the extra pair shows synapsis (and probably chiasma formation). This means that (1) the extra pair contains essential homologous genic matter to ensure effective pairing, (2) the extra pair came from homologous segments of a homologous pair of parent chromosomes, and (3) the extra pair carries genic matter essential to the survival and success of the plant. This is why its transmission is effected by a genetic mechanism. The failure of this mechanism ultimately leads to the formation of inviable meiotic products.

The behavior of this extra pair during cell division is of interest. The incidence of fragments within chromosome complements has been reported, both in nature and in experiments, by several workers. In experiments, ionizing irradiation has been employed to effect fragmentation of chromosomes. The broken ends of the fragments may then either heal, rejoin by restitution, or rejoin with broken ends of other chromosomes (Lea, 1946; Hair, 1952; Giles, 1954). If restitution is to occur, it occurs immediately after the breakage and is dependent on oxidative enzyme metabolism (Wolff & Luippold, 1955). The extra pair of chromosomes (fragments) in *U. altissima* has healed broken ends in somatic cells and hence behaves as a normal autosomal pair during mitosis. During meiosis, however, the ends either remain healed and the pair forms a bivalent of its own, which becomes excluded

during polar movement, or the broken ends become reactivated and thereby the pair associates with other chromosome pairs. Such a behavior has not been reported earlier. Rees (1958), however, working on *Scilla,* reported the behavior of chromosome fragments in pollen mother cells. In *Scilla,* the fragments become attached either to their parent autosomes or to any other chromosomes, depending on the relative distance of the fragments to one or the other. The attachment may be synchronous or asynchronous. Synchronous attachment must take place within a sticky matrix. As no sticky matrix was found in *U. altissima,* there was no evidence to suggest that the association of the extra pair with the second large autosomal pair was anything but synchronous. Hence the situation in *U. altissima* differs from that of *Scilla* in that it shows clear evidence of a genetically controlled mechanism of association, thereby stabilizing the resultant genetic system to ensure a high percentage fertility of sexual reproduction. This is in keeping with the suggestions of Blackwood (1956) and Rutishauser (1956).

It will be interesting to find out what initiates the mechanism of healing and reactivation of the broken ends of the fragments and the second large autosomal pair during mitosis and meiosis. It is likely that the stage of development at which the dissociation occurs is the interphase between the end of spore formation and the beginning of spore development, while the reactivation of the broken ends would take place at the onset of meiosis in the sporocyte. Since flowering and sexual reproduction are generally controlled hormonally, these events are likely to be part of the effects of the hormonal control, which in itself is genetic. This allows repetition of this event whenever necessary.

In this case, the extra pair of chromosomes should be regarded as fragments that are genically essential to the survival and success of the genetic system of the plant species. By this fragmentation, an otherwise somatic number of $2n = 20$ has become $2n = 22$. This $2n = 22$ should, however, be seen as $2n = 20 + 2$ ff.

The evolution of this new number may be connected with the morphological differentiation and differential ecological preference that have led to the recognition of *U. altissima* sensu stricto from its relatives *U. gigantea* and U. *viridula* Baker in the *U. altissima* complex (Oyewole, 1975a).

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